STUDIES ON THE TRANSMISSION, EFFECT, AND CONTROL OF 2 VIRUSES ON LINUM USITATISSIMUM L.

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INTRODUCTION

Aster yellows was first described in flax in California in 1945 (24). This disease is caused by the aster yellows virus (AYV) and is transmitted principally by the six-spotted leafhopper (Macrosteles fascifrons Stal.). Other insect vectors of AYV and the known host range of the virus consisting of nearly 400 different plant species has recently been summarized by Wallis (25).

It was not until 1954 that a report of aster yellows of flax was made for the Upper Midwest (6) and the Canadian Prairie Provinces (21). In 1957 a severe epiphytotic of aster yellows occurred in the flax crop in Minnesota, North and South Dakota and adjacent parts of Canada. It was estimated that losses of between 15 and 20 per cent of the flax crop were caused by this disease (8, 22, 23). In addition, a new virus disease of flax, crinkle, caused by the oat blue dwarf virus (BDV), was prevalent during this season (3, 9). Since 1957, however, the incidence of aster yellows of flax has been low in the major flax growing areas of Minnesota, although fields with 10 per cent aster yellows were reported in 1958 and some fields with 20 per cent were found in 1959. Similarly, the incidence of crinkle in commercial flax fields has been low since 1957.

An extensive review of literature and corroboratory work on the transmission of aster yellows has been made recently and therefore need not be presented here (5).

Reports of varietal differences of various crops to AYV have been discussed recently by Putt and Sackston (21). In their studies certain inbred lines of sunflower (Helianthus annuus L.)

were found to be resistant to aster yellows. Control of aster yellows of flax by means of introduction of resistant varieties was proposed by Martin et al in 1961 (19). Martin based his suggestion on selections made within varieties from the World Collection of Flax. Unfortunately these flax lines were not highly resistant to AYV. Some reduction in the incidence of aster yellows has been achieved in other crops by control of the vector with insecticides (5, 12, 26). There have been no reports, however, of reduction in the incidence of aster yellows in flax as a result of insect control.

Lee and Robinson (16) have shown in field experiments that flax was the least preferred host of aster, lettuce, parsley and carrot for the six-spotted leafhopper. Giralami (10) who studied the anatomical effects of AYV on flax found that tissue degeneration of sieve tubes followed by cells next to the sieve tubes was greatest at the location of leaf insertion.

The work reported here deals with the effects of AYV and BDV on flax, the possibility of developing resistant or tolerant varieties of flax to aster yellows and control of the vector by means of systemic insecticides.

MATERIALS AND METHODS

Leafhoppers used in these studies were collected from various crop and weed sources in the field with insect sweep nets. Collections were most successful during periods of low relative humidity and wind velocity. Quick field identification was made on the basis of the yellow-green color of the insect and movement in a zig-zag manner on a transparent surface such as an automobile window. Identification of the six-spotted leafhopper (Macrosteles fascifrons Stal.) was determined from the classification scheme of Beirne (4).

Colonies of insects were reared on oat seedlings at 24-26°C in cages consisting of aluminum frames enclosed with a 40 mesh nylon screen. The cages were 80 cm high and 45 cm square with glass on the top and front (Fig. 1). Three hundred-watt bulbs suspended above the cages were used to maintain a photoperiod of not less than 12 hours during the winter months. Insect transfers were made in the headhouse of the greenhouse away from other experimental material.

To obtain viruliferous colonies of insects, virus-free leafhoppers were placed on AYV-infected asters or Nicotiana rustica for 5 days and then transferred to oats for 15 additional days for completion of the incubation period. Generally, 25-30 per cent of the insects handled in this manner transmitted AYV. For acquisition of the oat blue dwarf virus (BDV), insects were fed on diseased oat plants for similar lengths of time and retained on oats or asters through the incubation period.

Fig. 1. A cage used for rearing the six-spotted leafhopper in the greenhouse.

Inoculation of flax varieties was principally by 2 means: either 2 seedlings were exposed to 5 leafhoppers from a colony of viruliferous insects for 2 days by use of chimneys or 50 seedlings were exposed to 100-125 leafhoppers for 2 days in greenhouse cages.

When an individual insect was tested, it was usually confined to the test plant by a glass chimney, covered with 60-80 mesh organdy cloth attached to the chimney by a rubber band (Fig. 2). By this means insects were indexed for the presence or absence of virus, or small lots of insects were placed in these chimneys with a suitable host plant for completion of an incubation period. Chimneys also were used as a means of exposing small lots of insects to detached plant parts in order to acquire the virus. For example, virus-free insects were exposed to detached dodder stems that had been removed from virus diseased plant tissue and placed on moist cheesecloth inside the chimney.

Viruliferous leafhoppers were fed on specific plant parts by 1 of 2 means:

1) Cellulose casings, 36 mm in diameter were cut to the desired lengths and placed over the plant part to be inoculated with virus. The bottom of the cellulose casing was then tied shut around the plant so that the insects could not escape. Insects were dropped carefully into the chamber by an insect aspirator and the top was closed with a cotton cord, thus providing a convenient cylindrical insect chamber around the flax plant.

2) The other method for leaf inoculation consisted of the use of small insect chambers made from extruded plexiglas tubing 1.5 cm in diameter and 2 cm long. These chambers described previously

Fig. 2. Glass lamp chimneys used for inoculation of individual or small numbers of flax seedlings with AYV or BDV by leafhoppers.

(13) were modified by cutting a slit in one end for inserting a flax leaf into the chamber (Fig. 3).

Inoculated flax seedlings were incubated in greenhouses held at various constant temperatures, i.e., 18, 24, and 30°C. For most of the experiments, the temperature was about 24°C. Although the greenhouse temperature is known to fluctuate, the mean temperature over a period of several days will closely approximate the indicated temperature.

Larger wooden framed cages, 100 x 60 x 76 cm were used in field inoculation experiments. Viruliferous insects reared in the greenhouse were introduced into these cages at times indicated for the experiment.

Phorate[1] and Disyston[2] applications to farmers' fields were made with field fertilizer spreaders or with a cyclone grass seeder. Band applications of phorate were made by drilling a strip of pre-weighed 10 per cent granular into rows 1.5 cm deeper than the seed. For seed treatment with phorate a 44 per cent active phorate composition in a carbon base was coated on the seed with a methyl cellulose slurry.

The strain of AYV used in these studies was found to infect celery (Apium graveolens L.) and therefore could not be considered the eastern strain of AYV (15) but did not infect alsike clover (Trifolium hybridum L.) or red clover (Trifolium pratense L.),

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[1] 0,0 diethyl S-(ethylthio) methylphosphorodithioate or Thimet, supplied by American Cyanamid Company.

[2] 0,0 diethyl S-2-(ethylthio) ethylphosphorodithioate supplied by the Chemagro Corporation.

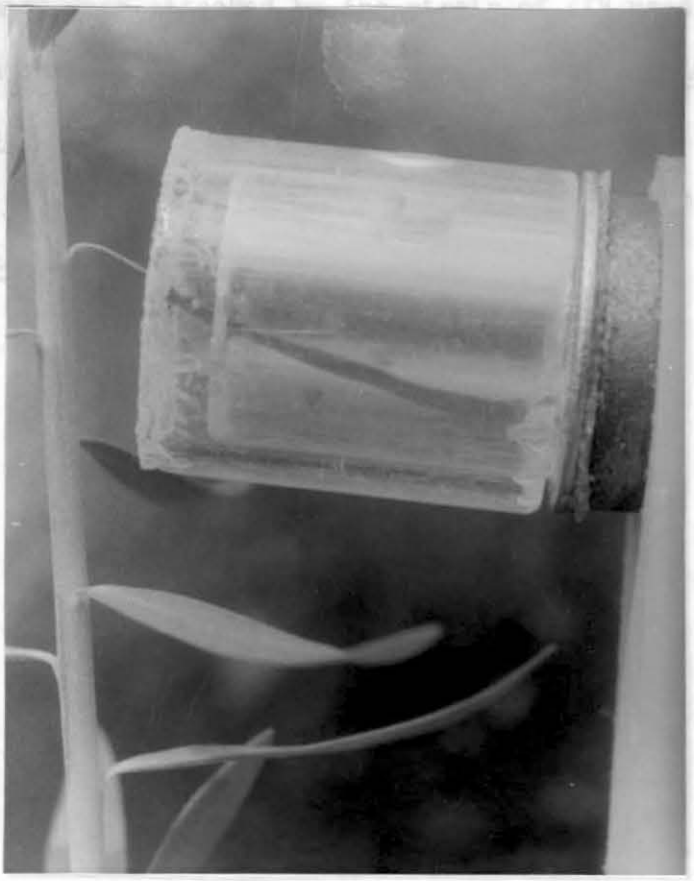

Fig. 3. Small insect chamber used for inoculating a specific area of a flax plant with virus by the insect vector.

hosts which have been described for the western strain (11). Other hosts which were attacked by the strain of AYV used were as follows: China aster (Callistephus chinensis Nees.), flax (Linum usitatissimum L.), Nicotiana rustica L., barley (Hordeum vulgare L.), carrots (Daucus Carota L.), and 2 common weed hosts, ragweed (Ambrosia bidentata Michx.), and fleabane (Erigeron spp.).

RESULTS

Symptoms of Aster Yellows and Crinkle of Flax

Aster Yellows

Symptoms of aster yellows of flax in the field

Generally the flax plants are yellow-green and have numerous adventitious branches with aborted flowers. Individual diseased flowers have bright yellow-green sepals which extend at right angles from the base of the pedicle forming a 5-merous "starflower" such as shown in Fig. 4. Sepals from healthy flowers tightly encase the corolla (Fig. 5). Petals, anthers and stamens in diseased flowers are chlorotic and rudimentary whereas the pistil, although abnormal, may or may not continue to develop. Elongation of the pistil is more characteristic of flax flowers from artificially diseased plants in the greenhouse.

The flax inflorescence is an indeterminate cyme and frequently diseased plants in the field are only partially virus infected. That is, there are usually healthy and diseased flowers within the inflorescence. Another common variation is the development of one or more virus diseased tillers on an otherwise healthy appearing plant. Because of the striking symptoms, AYV diseased flax plants are easily detected in the field.

Symptoms of aster yellows in the greenhouse

In the greenhouse a greater range in aster yellows symptoms are encountered. Diseased seedlings which are rarely observed in the field are easily obtained by artificial inoculation. On seedlings, the youngest leaves are chlorotic and the apical

Fig. 4. A close-up of a flax blossom infected with aster yellows.

Fig. 5. Symptoms of AYV on flax. The upper center flower is normal with the exception of the two petals removed for photographic purposes. The lower left flower became diseased after blooming, whereas the flower on the lower right was diseased before blooming.

portion of the plant is tilted or inclined slightly. At first the subsequent internodal elongation is 3 to 4 times greater than normal and cleared or translucent. Finally as new leaves appear internodal elongation decreases and with each new leaf also a gradual decrease in size. The ultimate appearance of a diseased seedling can be likened to a sharply angled cone.

When larger seedlings or plants become infected, symptoms not unlike those which appear in the field are common. However, pistil elongation as shown in Fig. 6 is more prevalent. Frequently the pistil in the diseased flower elongates several centimeters above the receptacle and appears macroscopically as a continuation of the stem (Fig. 7). At the apex of the transformed pistil there are usually 5 yellowed leaves and 5 branchlets and finally these branchlets have 5 yellowed leaves at their apices. Disease symptoms, however, vary greatly, depending on the stage of development of the plant at the time of infection.

Another symptom of aster yellows of flax is a "shepards crook" that has been found in only 1 flax line, Iso. 419[3]. Shepards crook appears somewhat like flax wilt, i.e., the plants become yellow and peduncles bend over in one tiller or the main trunk following infection of another tiller or portion of the plant (Fig. 8).

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[3] A flax line obtained from a genetic study. See Culbertson, J. O., V. E. Comstock, and R. A. Frederiksen. 1960. Further studies on the effect of seed coat color on agronomic and chemical characters and seed injury in flax. Agron. J. 52:210-212.

Fig. 6. An AYV infected flax flower. Note the "transformed" and elongated pistil.

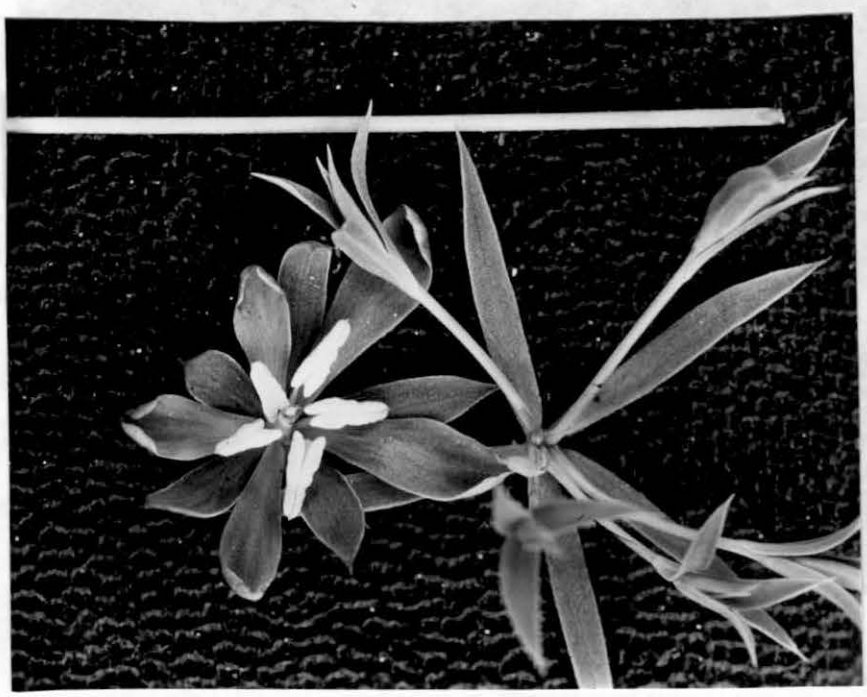

Fig. 7. The phyllody of a flax flower caused by AYV infection: left: modified sepals, petals, and anthers; top: pistil transformed into stem; right: whorl of leaves, stems, and additional leaves borne on stem (transformed pistil).

Fig. 8. "Shepards crook" of flax caused by AYV; healthy on left, diseased plant on right.

Symptoms of crinkle of flax

A new virus disease of flax, crinkle, was reported by Frederiksen and Goth in 1959 (9). Subsequently, Banttari (1) and Banttari and Frederiksen determined that the virus causing crinkle in flax was identical to the oat blue dwarf virus (BDV) (3). Macrosteles fascifrons, the vector of AYV, was found also to transmit BDV (3). Flax plants infected with this virus are slightly stunted (Table 1) with irregular protrusions or small enations located on the principal lateral veins of the leaf (Fig. 9). These enations are commonly observed on the sepals of flax blossoms (Fig. 10). Since crinkle diseased flax plants bloom more or less normally and the most characteristic symptom is the small enations, these plants are very inconspicuous in the field.

Double infections of flax with AYV and BDV

Plants with both aster yellows and crinkle were infrequently observed in the field and generally these plants would have 1 virus disease in the main trunk and the other in a tiller. Occasionally, however, tillers of some plants would be infected with both viruses. In the greenhouse, plants infected with the combination of viruses had hypertrophied apical growth, enations as caused by BDV, and yellowing of leaves and stem tissue as normally caused by AYV (Fig. 11). These symptoms are easily differentiated from symptoms caused by the individual viruses. The characteristics of plants infected with both viruses were essentially the same when artificially inoculated in the greenhouse as in the field. Variation in symptoms due to time of infection of 1 virus in relation to the other will be discussed later.

Table 1. Average height in centimeters of greenhouse and field grown flax plants infected with BDV as compared to paired plants which were virus-free plants.

Plants	Plant height in cm	
	Greenhouse[a]	Field[b]
Virus-infected	55.6	46.5
Virus-free	79.4	63.5

[a] Based on 15 comparisons

[b] Based on 110 comparisons

Fig. 9. Small enations or protrusions on flax leaves caused by BDV.

Fig. 10. Enations on sepals adjacent to a flax capsule caused by BDV.

Fig. 11. Symptoms typical of a flax seedling infected with both AYV and BDV. Note the small enations on the leaves typical of crinkle and the apical clearing and elongation caused by AYV. The apical enlargement of the stem, however, results only from the combination of the 2 viruses.

Transmission of Aster Yellows and Blue Dwarf Viruses

Transmission of AYV and BDV to flax by dodder

Although the aster yellows virus was thought to cause "aster yellows of flax", prior to this work, no proof by recovery of the virus from flax in the Upper Mississippi Valley area had been obtained. Therefore as soon as virus-free insects were available, numerous attempts were made to recover AYV from diseased flax by feeding virus-free leafhoppers on varieties at different stages of plant growth on plants which had been diseased for varying lengths of time. In none of these trials was AYV recovered from flax, although AYV was recovered by insects from diseased asters which were included as a control.

The first transfer of AYV from flax to flax was achieved by the use of dodder (Cuscuta sp.)[4]. Flax plants infected with AYV were exposed to dodder stems from virus-free flax plants. Subsequently the dodder stems became attached to the infected flax plants. The dodder, now possibly infected with aster yellows, was attached to other virus-free flax plants and later, in most cases, these plants developed aster yellows. No aster yellows appeared on virus-free dodder or virus-free flax plants. Although serial transfers were made, for each transfer of virus to a successive generation of flax, new virus-free dodder was needed as this species of dodder is susceptible to aster yellows. Dodder infected with

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[4] The dodder used in these studies was found as a contaminant in an imported Turkish flax variety by Dr. V. E. Comstock, Crops Research Division, A.R.S., Department of Agronomy and Plant Genetics, St. Paul, Minnesota, and has been tentatively identified as C. Epilinum Weibe.

aster yellows was characterized by dark color, poor vigor and phyllody. Growth of dodder on an AYV diseased aster is shown in Fig. 12. Diseased dodder produced no seed.

Similarly, dodder was used to transfer BDV from flax to flax. Whereas it required over 1 month to produce aster yellows in flax by dodder, two to 3 weeks were sufficient to transfer BDV and cause crinkle symptoms in flax. In addition, the dodder did not appear to be affected by the virus as was the case of AYV. Serial transfers such as done in aster yellows were also done for crinkle. In addition, dodder growing on blue dwarf oat seedlings was attached to flax causing crinkle. Subsequently, virus-free dodder stems were attached to these diseased flax plants and after several days growth reattached to oat seedlings. Three out of 20 of these attempts proved successful in transferring the blue dwarf virus from oats to flax and back to oats again. As a final check virus-free leafhoppers fed on these oat seedlings with blue dwarf were found to be transmittors of BDV.

Several attempts to recover aster yellows and crinkle from dodder by insects were made in the course of these investigations. Johnson had previously shown that AYV could be recovered from diseased dodder (13).

Dodder, which had been growing on virus-infected hosts, was detached and exposed to virus-free leafhoppers for 1 to 2 day periods. In several cases BDV was recovered from dodder; however, AYV was obtained only rarely (Table 2). Aster yellows virus and BDV were both recovered on 1 occasion from dodder which had grown on flax infected with both viruses.

Fig. 12. Comparative growth of dodder on healthy aster (left) and AYV diseased aster (right). A similar response occurs when dodder is grown on virus-free and AYV diseased flax.

Table 2. Recovery of AYV and BDV by leafhoppers from dodder stems grown on hosts infected with aster yellows, crinkle, or both viruses[a]

Dodder stems from	Number of recoveries attempted[b]	Number of successful virus recoveries	
		AYV	Crinkle
Aster yellows and crinkle flax	6	1	1
Crinkle flax	9	0	5
Aster yellows aster	1	1	0
Virus-free aster	3	0	0
Virus-free flax	9	0	0

[a] Dodder stems were removed from their plant hosts and placed under glass chimneys on moist cheesecloth and exposed to virus-free leafhoppers.

[b] For each attempt to recover the viruses, 10 leafhoppers were used.

Transmission of AYV and BDV by Macrosteles fascifrons

Inoculation of flax with AYV by Macrosteles fascifrons.--

Initially, experiments were made to determine whether flax could be artificially inoculated with AYV by viruliferous[5] insects. Inoculations were made by enclosing plant parts with cellulose casings in which 10 insects were placed for 2 days. Flax plants at various stages of plant growth were inoculated by this method.

The controls were identical to the other treatments except virus-free insects were used. In preliminary tests with individual plants, it was found that flax was susceptible to the strain of aster yellows used and that seedlings 15 to 20 cm tall and plants in the blooming stage were susceptible. Almost without exception, the inoculated plants succumbed to the disease. Plants exposed to virus-free insects did not develop aster yellows.

Comparison of flax and aster seedlings in inoculation trials and speed at which insects could inoculate flax with AYV.--

Comparisons were made between the efficiency of inoculation of aster and flax seedlings. Insects from a viruliferous population were fed individually on flax or aster seedlings for 1, 2, and 3 day periods by encaging the insect over the plant by lamp chimneys. An insect that fed on both an aster or flax seedling and was transferred 1, 2,

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[5] The term viruliferous implies that these insects were previously exposed to a virus source. Every insect within a population will not become a vector. The percentage of actual vectors within a population depends on the source of virus and the duration of time for which the insect was in contact with the virus source. Generally less than 50 per cent of the insects are vectors; an average of about 30 per cent or less was common.

or 3 days later to the other plant for the same length of time was studied. Sixty-seven per cent of all inoculations made on flax were successful, indicating a high percentage of the insect population were vectors of AYV. There was no difference in the percentage of viruliferous insects as assayed on flax in the 1, 2, and 3 day feeding periods (Table 3). However, there were fewer infections made by these insects for any of the 3 different feeding periods on asters as compared to flax. The fewest number of successful infections occurred with the 1 day feeding period on asters. Although the number of comparisons that were made were small (18 and 36) the evidence strongly indicates that flax is as susceptible, if not more so, to receiving aster yellows from the individual insect vectors than aster seedlings.

A number of observations to determine whether viruliferous insects could transmit AYV to flax in 5 minutes or less were also done. Feeding time in these observations was based on the time that the insect actually appeared to begin feeding within the plant. Three successful infections were obtained from 10 periods of 5 minutes each from insects known to be vectors of AYV. In 1 instance, a single insect transmitted AYV to flax in a 1 minute feeding period. Although the number of these observations were few, the fact that an individual insect can transmit the virus in 5 minutes or less is indicative of the difficulties that may arise in attempting to reduce aster yellows by vector control. That is, the insecticide may not have eliminated the vector prior to virus transmission.

Table 3. Percentage of AYV infected aster or flax seedlings when each were inoculated with the same individual leafhopper for 1, 2, or 3 days

Feeding period in days	Number of insects tested	Percentage of plants infected	
		Aster	Flax
1	36	39	67
2	18	50	67
3	18	50	67

Transmission of BDV to flax by Macrosteles fascifrons.--Transmission of the crinkle virus to flax was accomplished principally at first by collecting insects from the field and feeding them on healthy flax seedlings. An incidence of 5 to 10 per cent of the plants with crinkle was not uncommon when 200-300 leafhoppers were fed on 50-100 flax seedlings for several days. However, much difficulty was experienced in attempting to isolate the virus from flax by vectors. It was not until the discovery that crinkle of flax and blue dwarf of oats were caused by the same virus (3) that studies on the crinkle disease of flax could be done in the greenhouse.

Recovery of AYV from flax.--Although AYV and BDV were found to cause aster yellows and crinkle in flax, respectively, it seemed important from the standpoint of epidemiology to determine the efficiency of recovery of either or both of these viruses from diseased flax. If flax is as good a virus source for acquisition by the vector as it is susceptible to AYV, aster yellows epidemics in flax may be more important and frequent than if the virus was difficult to acquire by the vector.

Although previous attempts to recover AYV from flax by the six-spotted leafhopper had failed, the same technique employed in obtaining AYV from detached dodder stems was applied to flax. By removing apical portions of flax plants with AYV and exposing them to virus-free leafhoppers, AYV in flax was recovered and transmitted to flax. In the first experiment only 3 out of over 100 insects recovered the virus from flax. In addition, the incubation period was found to be nearly 40 days at 24°C. The initial failure to

recover AYV from flax may have been caused by an insufficient incubation period for the insect vectors. In addition, the detached plant technique forced the insects to feed on only diseased portions of the flax plant.

To determine whether different detached parts of the apex of the flax plants differed in effectiveness as a virus source, an experiment was made comparing: a) the upper $\frac{1}{2}$ inch of the diseased flax plant; b) the next $\frac{1}{2}$ inch of stem tissue; and c) the leaves on the upper inch of the flax plant. Comparable parts from 3 diseased plants from the flax line Iso. 419 were used in each of the 3 replicates. Virus-free flax, virus-free aster, and AYV-diseased asters served as controls. For each treatment 20 virus-free insects were introduced into glass chimneys for virus acquisition for 2 days. Following the 2 day acquisition feeding, the insects were held on virus-free oat seedlings for an additional 8 days. Subsequently, the surviving insects were transferred in bulk to a new set of virus-free flax plants every 5 days. Thirty-five days after exposure of these insects to the diseased flax, individual insects from each group were placed on virus-free flax seedlings for 5 days. The apical $\frac{1}{2}$ inch of the flax plant proved to be a better source of AYV in flax than either the apical leaves or lower apical stem tissue (Table 4). These results indicate that a higher concentration of virus is available in the uppermost flax tissue, thereby permitting more efficient recovery of the virus by the six-spotted leafhopper.

Table 4. Percentage of leafhoppers transmitting AYV after feeding on various portions of flax and asters with and without AYV

Host	Plant part tested	Percentage of vectors[a]
Flax	Apical leaves, from first inch	15
Flax	Top $\frac{1}{2}$ inch of stem tissue	30
Flax	Second $\frac{1}{2}$ inch of stem tissue	10
Flax	Aster (leaves)	58
Aster	Virus-free flax	0
Aster	Virus-free aster leaves	0

[a] Based on the surviving insects from a total of 60 used for each treatment.

Recovery of BDV from blue dwarf oat plants.--One hundred leafhoppers were placed on 3 oat plants infected with BDV. After 5 days 33 surviving insects were removed and placed on 33 individual flax plants. Eight of the latter contracted crinkle. Crinkle appeared first in 11 days and the last of the seedlings acquired virus symptoms 9 days later. One hundred virus-free leafhoppers, reared on virus-free oats, were placed on 10 flax plants for 5 days. None of these flax plants developed crinkle.

Subsequently, a larger population of virus-free insects were placed on oat plants with blue dwarf and sampled periodically to determine the acquisition efficiency of the virus. Ten insects were removed in each sampling. Although the sampling of the population was small, the acquisition of the virus by individual insects from the infected oat plants was readily accomplished. The percentage of leafhoppers with the virus did not increase when the insects were exposed to the infected blue dwarf oats for more than 4 days.

Recovery of AYV and BDV from flax infected by both viruses with insects.--Aster yellows virus and BDV were recovered from flax with both aster yellows and crinkle by feeding virus-free leafhoppers on detached apical portions of flax plants. Although the efficiency of virus recovery was low, it is apparent that both viruses were present in these diseased plants. Only the blue dwarf virus was recovered from plants with crinkle as indexed on flax and only AYV from those plants with aster yellows. However, when the plants were infected with both viruses either one or the other or both were recovered (Table 5).

Table 5. Number of successful recoveries of AYV and BDV by the six-spotted leafhopper from aster yellows and crinkle flax

Virus infection[a]	Number attempted	Number of successful recoveries[b]	
		AYV	BDV
AYV and BDV	18	2	6
AYV	9	2	0
BDV	9	0	1
Virus-free control	9	0	0

[a] All recoveries were from detached apical virus-diseased flax plants.

[b] Successful recoveries were determined by transmission of the viruses by the leafhopper to flax.

Double infection of flax with AYV and BDV.--It was determined on the basis of field observations that BDV did not protect the flax plant from infection with AYV (page 38). In addition, 2 greenhouse experiments were made to determine if either BDV or AYV would afford protection against infection by the other within individual Marine flax plants. Viruliferous populations of leafhoppers with AYV or BDV were obtained by exposing the insects to AYV diseased aster plants or oat plants with BDV. Plants exposed to insects with 1 virus were then held for 2, 4, and 8 days before exposure to insects with the other virus. Plants exposed to only 1 virus and then subjected to the proper number of virus-free insects at the appropriate time served as controls. Each treatment was replicated 5 times in the first experiment and 4 in the second.

It was determined at the onset of the experiment that 20 and 40 per cent, respectively, of the insect populations were actually vectors of BDV and AYV. Therefore, 10 insects were used for each inoculation of BDV and 5 for the AYV inoculation.

Unfortunately the number of control plants successfully inoculated with AYV were considerably fewer than those successfully inoculated with BDV. However, symptoms caused by infection of both viruses appeared at least once out of 5 for all the inoculation treatments except when the AYV inoculation followed the BDV inoculation by 8 days.

In the second experiment both viruliferous populations had approximately 30 per cent actual vectors; therefore, the number of insects used for each inoculation was limited to 5.

Prior inoculation with AYV did not inhibit development of the BDV (Table 6). Two and 4 day previous inoculation with BDV did not prevent the flax plant from developing aster yellows, as symptoms of both viruses were present in 1 flax plant.

Plants inoculated with BDV 8 days prior to inoculation with AYV did not develop symptoms typical of double infection. Symptoms of AYV were notably depressed and the plants were markedly stunted. The most extensive hypertrophy was detected in flax seedlings infected with BDV 4 days later than AYV.

Although symptoms caused by BDV infection of flax appear on the average 4 days sooner than symptoms caused by AYV, in these studies the time that it took for symptoms to appear for either virus was not influenced to any extent by previous inoculation with the other.

The presence of both AYV and BDV in some of these plants was demonstrated by the recovery of both of the viruses with insects from detached apical portions of the infected plants (note Table 5).

Dual transmission of AYV and BDV by individual leafhoppers.--Experiments were made to determine whether single leafhoppers could transmit both AYV and BDV simultaneously to individual flax plants. Two hundred AYV viruliferous leafhoppers were divided into 2 groups: 100 placed on virus-free oats and the other 100 on oats infected with BDV. Similarly, 200 BDV viruliferous leafhoppers were fed on asters with and without AYV infection. Virus-free insects were then fed on the same virus sources and virus-free insects on both virus-free oats and asters. There were, thus, a total of 8 vector-host combinations.

Table 6. Virus symptoms on Marine seedlings caused by inoculation with AYV, BDV, and a combination of AYV and BDV

Latent period		Symptoms produced by inoculation of			
days[a]	Replicate	AYV	BDV	AYV before BDV	BDV before AYV
2	1	A[b]	C	B*	B
	2	A	-	B*	A
	3	A	C	A	B
	4	A	C	A	B*
4	1	A	C	A	C
	2	A	C	B	A
	3	A	C	B**	C
	4	A	C	B**	B
8	1	A	C	B	B-
	2	A	-	B	B-
	3	A	C	B	B-
	4	A	C	A	C

[a] The number of days following 1 inoculation before re-inoculation with another virus. In the case of 1 virus inoculation, virus-free insects were fed on the plants as a control.

[b] The symptoms which appeared were A, aster yellows; B, both aster yellows and crinkle; and C, crinkle. When no symptoms appeared, a "-" is used. Variations in symptoms caused by infection of both viruses are noted as B*, some hypertrophy, B** extensive hypertrophy, and B- stunting.

Unfortunately, a high mortality of the leafhoppers occurred in some of these vector-host combinations because of insecticides used in the greenhouse where these tests were being made.

From AYV viruliferous leafhoppers that were fed on a BDV source 22 transmitted AYV and 3 of these insects transmitted both AYV and BDV. Conversely, of the insects originally BDV viruliferous that were fed on an AYV diseased aster plant, 7 survivors transmitted BDV and 1 insect transmitted both viruses.

When this experiment was repeated, the mortality of the leafhoppers was again extremely high. This time death of the insects was presumably caused by a fungus, Beauvaria sp., infestation which may have been aggravated by unseasonably high temperature and high humidity at the time of the experiment. For this experiment, there were only 39 insects which survived that had fed on plants with both viruses. Slightly over 23 per cent of insects transmitted BDV whereas 6 per cent transmitted AYV. Therefore, on the basis of probability less than 1 dual virus transmitting insect would be expected; however, 3 were observed. Although the number of successful transmissions was small, there did not appear to be any interference on the part of 1 virus to inhibit the transmission of another by the same vector. Over a period of 4 years over 1000 insects collected in the field were individually indexed for the presence of virus. In 1961 1 insect was found that transmitted both viruses.

Certain ecological factors affecting aster yellows and crinkle of flax

The effect of stand on the incidence of aster yellows and crinkle in Marine flax.--Three rates of sowing 4, 15, and 46 plants per foot were tested in all possible combinations with 2 row spacings, 6 and 12 inches apart in the row. This field experiment was replicated 4 times and seeded on 2 dates, April 29 and May 24, 1957. No data were obtained from a third planting because severe injury caused by feeding of the tarnished plant bug (Lygus spp.) obscured the symptoms caused by both AYV and BDV.

The incidence of aster yellows and crinkle was lower in the higher plant populations. There was an average of 15 per cent of the plants infected when there were 46 plants per foot, whereas 63 per cent of the plants were infected with AYV when there were only 4 plants per foot at the first seeding date (Table 7). Incidence of aster yellows was similar also in the late planting. The average percentage of plants infected with AYV increased slightly in the second planting although the difference between populations in percentage of virus-infected plants decreased. The increase of an average percentage of infection of 4 per cent for AYV in the second seeding date was not statistically significant.

For crinkle, differences in percentage of plants infected between the 3 rates of seeding and between dates were statistically significant. There was a higher incidence in the percentage of plants infected with BDV in the second seeding, indicating that a higher percentage of vectors transmitted BDV than AYV as the season progressed. This can be assumed since the same vector transmits both viruses.

Table 7. Percentage of plants with aster yellows (AYV) and crinkle (BDV) in Marine flax when seeded at different rates on 2 dates at St. Paul in 1957

Plants/foot	Virus infection in per cent[a]			
	Early seeding		Late seeding	
	AYV	BDV	AYV	BDV
4	63.4	20.0	52.1	63.6
15	38.3	11.2	40.0	61.1
46	15.0	7.2	20.2	30.6
Average	22.3	8.9	26.3	39.4

[a] Based on the average infection in 4 replicates of rows 4 feet long.

Crinkle infected plants noted in this nursery were tagged and later observed for aster yellows. Twenty-two per cent of all BDV infected flax seedlings became diseased with AYV, which, on the average, was the overall incidence for aster yellows in the nursery. Flax plants with crinkle can be observed in the field frequently 1 week or longer before flax plants with aster yellows are observed. It is therefore possible that many of the plants that were infected with crinkle may have been inoculated with AYV prior to BDV. Apparently neither virus protects against infection from the other. (Note: Double infections of flax with BDV and AYV in the greenhouse, page 34)

The spacing between rows had little effect on the incidence of either virus; however, there was a slight, but consistent trend for a higher incidence of virus in those rows spaced 1 foot apart as compared to those rows spaced 6 inches apart.

Temperature during virus incubation period in the plant.-- Since flax plants with crinkle appeared before aster yellows could be observed in the field, it is possible that the response may be due to temperature. It would probably not be due to the presence of BDV prior to the AYV since the first appearing insects transmit both crinkle and aster yellows. This has been ascertained by indexing individual insects from the field each spring for a number of years. The duration of the incubation or latent period in hosts inoculated with aster yellows is shortened at higher temperatures (18); however, the incubation period of BDV in flax may not be greatly influenced by temperature and therefore symptoms may appear in less time.

Experiments were made in the greenhouse to determine the effect of different temperatures on the length of the incubation period of flax inoculated with BDV and AYV. Inoculations were made on flax seedlings with 5 viruliferous insects at approximately 24°C for 2 days. The inoculated seedlings were then placed at the desired temperatures. As it can be noted in Table 8 there was a pronounced effect of temperature on the time required for symptom appearance for AYV. At a constant temperature of 18°C the incubation period is nearly twice as long, on the average, as it is at 30°C. The duration of the incubation period was based on observing an average of about 600 inoculated flax seedlings of several varieties at each temperature.

Following inoculation with BDV, flax seedlings develop crinkle in about 9 days irrespective of temperature, 18, 24, 30, or 32°C. For crinkle, many fewer plants of 1 variety, Marine, were studied; however, in this case, the temperatures were controlled by special thermostatically regulated plexiglas greenhouse incubators. Temperature variation in these chambers throughout the experiment was usually less than $\pm 2^{\circ}$. The lack of response of BDV to these various temperatures in all likelihood may account for the earlier appearance of crinkle as opposed to aster yellows in flax in the field.

Table 8. Incubation period in days for AYV and BDV in flax seedlings inoculated at various greenhouse temperatures

Temperature °C	Days for symptoms to appear			
	Aster yellows		Crinkle	
	Mean[a]	Range	Mean[b]	Range
32	-	- [c]	9.5	9-13
30	10.5	8-15	9.0	9-9
24	13.1	9-21	9.0	8-10
18	19.2	14-29	9.5	8-12

[a] Based on about 600 plants grown at each temperature.

[b] Based on an average of 6 plants at each temperature.

[c] No test.

Agronomic effects of aster yellows and crinkle in flax

Yield losses caused by aster yellows and crinkle.--

Reduction in seed yield of flax infected with AYV has been observed (7, 8). The following observations further augment and delimate in part the effects of these viruses on the number of seeds per boll, bolls per plant, and plant yield.

Aster yellows.--Thirty infected plants of B5128 (C.I. 980) and 30 infected plants of Marine with symptoms on June 26 were compared with the same number of plants with symptoms on July 6, 1957. Early infection nearly eliminated seed production of B5128 flax plants and severely reduced the yield of Marine (Table 9). Marine flowers several days earlier than B5128; therefore, its seed production probably was not affected as much as that of the later maturing flax variety.

In 1958 paired plants with and without AYV infection for the varieties listed in Table 10 were analyzed for seed yield. The average yield loss for all varieties was nearly 70 per cent. However, for specific varieties as Marine and Bolley, the losses were principally in numbers of seeds per boll and not in the number of bolls per plant. When considering Arny, a late maturing variety, AYV infection reduced both numbers of seeds per boll and bolls per plant. The other flax varieties in this test all matured later than Marine and Bolley and yielded less after infection, further suggesting that earlier maturing flax varieties are less severely damaged by AYV than those that mature later.

The effect of crinkle on yield of flax.--The yield of flax

Table 9. Number of bolls and seed yield of B5128 and Marine when infected with AYV at 2 different times during the growing season, 1957

Variety and time of infection	Average number of Bolls per plant	Seeds per boll	Total number of seeds per plant
B5128			
June 26[a]	1.6	.4	.7
July 6[b]	6.3	2.1	13.4
No infection	20.4	3.7	75.3
Marine			
June 26	3.2	2.4	7.8
July 6	9.6	2.8	27.0
No infection	20.9	5.6	116.6

[a] Shortly after blooming.

[b] Plants that had some boll formation.

Table 10. The average number of seeds per boll and bolls per plant from 6 flax varieties with and without AYV infection at St. Paul in 1958

Variety	Average number of seeds/boll[a]		Average number of bolls/plant[b]	
	Virus-free	AYV-infected	Virus-free	AYV-infected
Arny	6.1[c]	2.0	24.1	6.9
Bolley	6.7	3.2	18.7	20.6
B5128	7.3	4.8	30.3	16.8
Marine	7.3	4.8	21.6	23.7
Redwood	7.0	4.6	27.0	19.3
CI 1664	6.4	2.5	18.3	16.2
Mean	6.5	2.9	23.3	16.2

[a] The mean reduction in number of seeds per boll was statistically significant (P=.99).

[b] The mean reduction in number of bolls per plant was statistically significant (P=.95).

[c] An average of 52 plants was used to obtain these values.

plants infected and not infected with BDV was compared in 20 pairs of flax plants in 1958 and 1961 in the field in a heterogeneous flax nursery. The plants in the test were tagged prior to blooming during a 2 day period and allowed to remain in the field until harvest. Numbers of bolls per plant and numbers of seeds per boll were compared for the virus-free and diseased lines in 1958 whereas only total seed yield per plant was obtained in 1961. During the winter of 1960, 20 Marine flax seedlings with crinkle and a similar number of virus-free plants were grown to maturity in the greenhouse. The number of seeds per boll and the number of bolls per plant for each was determined.

Although the number of samples was comparatively small, there were consistent and statistically significant reductions in flax yield due to BDV infection (Table 11). There was a particularly striking reduction in the number of bolls per plant due to virus infection in the greenhouse grown plants. A reduction from 32.5 on the virus-free plants to 17.6 bolls on crinkle flax plants from the field was noted.

Germination of flax seed from aster yellows and crinkle flax.--When plump seed from either aster yellows or crinkle flax was compared to similar seeds produced on paired virus-free flax, there was little reduction in seed germination or seedling vigor. These observations on aster yellows were based on 360 plump seeds produced on 4 different lines in 1958 and seed from 18 different lines in 1959. For crinkle infected flax, comparisons were made from 20 flax lines in 1958 and 42 flax lines in 1959.

Table 11. The number of seeds per boll, bolls per plant and seed weight of crinkle flax plants and paired virus-free controls

Plant source	Virus-free	Crinkle
Field		
Number of bolls per plant, 1958[a]	32.5	17.6
Number of seeds per boll, 1958	7.0	5.6
Seed weight in grams per plant, 1961[b]	0.84	0.25
Greenhouse		
Number of bolls per plant, 1960[c]	37.2	7.3
Number of seeds per boll, 1960	6.8	5.0

[a] Based on 20 paired plant comparisons.

[b] Based on 30 paired plant comparisons.

[c] Based on 20 paired plant comparisons.

In no case did plants which grew from seeds produced on virus diseased flax plants develop virus symptoms.

Control Measures for Aster Yellows of Flax

Methods of evaluating flax varieties for resistance to aster yellows

Field selection under conditions of natural infection.-- Nine hundred seven lines from the world collection of flax and a few other lines were grown at the Agricultural Experiment Station, Rosemount, Minnesota, in 1957. The average incidence of aster yellows was over 15 per cent. Out of this material, 20 lines of flax were selected as having less than 3 per cent infection (Table 12). None were found which were immune to AYV.

In 1959, some natural infection of AYV was present at Grand Rapids, Minnesota. At Grand Rapids, Crookston, and St. Paul 10 lines of flax were grown in 2 replicated 18 foot rows of approximately 100 plants each. The percentage of plants with aster yellows for each variety was determined. A selection from the variety CI 1146 and Iso 419 had the fewest infections at Grand Rapids but were highest at St. Paul (Table 13). Unless methods of controlling and introducing artificial epidemics of AYV can be developed for use in the field, selections for resistant varieties will have to be done in the greenhouse.

Selection for aster yellows resistant flax in the greenhouse.-- Prior to testing flax varieties in the greenhouse, an experiment was made to determine a suitable technique for the inoculation of few plants of the susceptible variety Marine.

Table 12. Lines of flax in the world collection that had less than 3 per cent aster yellows at Rosemount, 1957

C.I. Number	Variety, cross or source country
674	Winona x Ottawa 770B F6
1020	Crepitans (Albertico, E978)
1125	Viking x (Bison x Rio) W18-36
1129	Atlas (Fiber)
1164	(Arg. 191 x Bison) x Viking x Bison
1175	Can 67-3901B
1178	No. 1276, N.D.
1236	10398/46 Argentina
1243	1045/46 Argentina
1259	10422/46 Argentina
1288	10456/46 Argentina
1317	India
1319	Turkey
1336	Bisbee
1364	Turkey
1376	Concurrent
1380	Hollandia
1381	Rembrandt
1399	Turkey
1446	Turkey

Table 13. Percentage of AYV infection present in 10 flax lines grown at 3 locations in Minnesota in 1959

Variety	Average virus infection at[a]		
C.I. number	Crookston	St. Paul	Grand Rapids
693	4.5	3.1	10.5
716	2.1	5.2	15.8
1130	9.5	5.0	17.0
1135	4.8	5.4	12.5
1140	3.5	2.1	18.5
1146	2.6	7.8	8.5
1259	3.3	4.7	10.9
1288	2.6	4.3	28.8
1380	4.5	2.0	11.5
Iso 419	2.5	5.8	8.7

[a] Average infection present in 2 18-foot rows of 100 plants of each variety.

Five insects from a population which was 35 per cent AYV viruliferous were placed on 2 or 5 flax seedlings for 3 or 5 days. This method involved the use of glass lamp chimneys and was replicated 6 times. Insect survival and numbers of infected plants in each pot were determined.

Pots containing 5 seedlings had about half as much infection as those with 2 plants per pot (Table 14). These results parallel the field data. The fewer the plants per given area, the higher the incidence of virus infection. Insect survival was slightly better when fewer plants were used, but there appeared to be no difference in survival in 2 or 5 days within the chimney inoculation chambers.

On the basis of this experiment it seemed plausible that varieties could be compared in greenhouse trials. Therefore, 14 flax varieties including several lines selected as somewhat resistant to aster yellows at Rosemount, Minnesota in 1957 and others as controls were compared. All inoculations were made by feeding 5 leafhoppers from insect populations 25 to 35 per cent AYV viruliferous on 2 flax plants per pot of each variety for 2 days at 30°C. Forty-eight plants of each variety were inoculated, 24 seedlings in the cotyledon stage and 24 seedlings, 10-12 cm tall. Following inoculation, 6 plants of each variety at each stage of growth were grown at 4 different temperatures: 18, 22.5[6], 24, and 30°C. Because of the size of the experiment, only half of the varieties were inoculated at 1 time.

Data were recorded on insect survival during the 2 day

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[6] The temperature in this greenhouse was held at 24°C during the day and 21°C at night.

Table 14. Average number of surviving leafhoppers and percentage of Marine seedlings with aster yellows following 3 or 5 day feeding period on 2 or 5 plants[a]

Feeding period in days	Number of plants per pot			
	2		5	
	Per cent AYV[b]	Insect survival	Per cent AYV	Insect survival
3	100	4.2	50	3.2
5	92	3.5	64	3.2

[a] Five insects were used for each inoculation. Thirty-five per cent of these insects were vectors of AYV.

[b] Based on an average of 6 replicates.

feeding period, percentage of plants with aster yellows, and the number of days required for symptoms to appear (incubation period).

There was no relationship between insect mortality and the flax variety inoculated, i.e., insect survival was similar on all varieties inoculated.

The differences among varieties in percentage of plants infected were not large. Redwood (CI 1130), a commercial variety that was diseased as severely as any other flax variety during the aster yellows epidemic of 1957, had the lowest percentage of infected plants in the first group of varieties tested. It is very likely that some differences among varieties were due to escapes, since most of the surviving non-infected plants, when re-inoculated, succumbed to the disease (Table 15). However, those few plants which escaped infection following the second inoculation were grown to maturity and harvested. The progenies of these lines were later inoculated with AYV.

Seedling size and temperature had little effect on the percentage of plants infected (Table 16), although there was slightly less virus infection in the plants incubated at 18°C. This may be accounted for in part since the duration of the experiment was 30 days and occasionally, based on more recent tests some plants have virus incubation periods of more than 30 days at 18°C.

Plant height and temperature, however, did have an effect on the length of the virus incubation period. There was a consistently longer virus incubation period in the larger plants and at the cooler greenhouse temperatures (Table 16).

The effect of 4 different temperatures, 2 seedling sizes at the time of inoculation, and 9 flax varieties (the second lot of

Table 15. Percentage of flax plants with aster yellows in 16 lines of flax inoculated in the greenhouse[a]

Variety CI number	Percentage of plants with aster yellows[b]
First group[c]	
302	68.8
303	58.3
674	64.6
1129	56.3
1130	41.9
1135	52.3
1135[d] (Marine check)	54.2
Second group	
320	81.3
1140	54.2
1146	72.9
1164	58.3
1236	60.4
1259	56.3
1288	55.3
1317	81.3
1135 (Marine check)	62.6

[a] Inoculations with AYV were made by feeding (25-35 per cent) viruliferous leafhoppers, 5 at a time, for 2 days on 2 flax plants at a time.

[b] Average infection of 48 plants of each variety.

[c] Because of the size of this experiment, 7 varieties of flax were tested at 1 time and 9 the next.

[d] A selection of Marine (No. 9) used as a control in all greenhouse inoculations.

Table 16. Percentage of plants infected with AYV and length of incubation period in days for 16 flax varieties grown at 4 temperatures

	Greenhouse temperature (°C)			
	18	22.5[a]	24	30
A. Effect of temperature on percentage of plants infected				
First group[b]				
Seedling	48.2	79.6	63.0	62.4[c]
Plants 10-12 cm high	63.0	59.3	72.2	75.9
Second group				
Seedling	47.8	60.5	57.8	73.8
Plants 10-12 cm high	54.2	51.1	54.3	56.3
Mean	53.5	63.1	62.3	67.5
B. Effect of temperature on virus incubation period				
First group				
Seedling	14.6	14.9	11.6	10.3
Plants 10-12 cm high	19.6	15.3	14.7	10.6
Second group				
Seedling	20.2	16.7	12.0	9.2
Plants 10-12 cm high	23.8	18.3	14.3	11.9
Mean	19.2	16.4	13.1	10.5

[a] Day temperature in this greenhouse was held at 24°C and 21°C at night.

[b] Because of the size of this experiment, 7 varieties of flax were tested at 1 time and 9 the next.

[c] Each figure is based on an average of 20 plants or greater.

varieties tested) on the length of virus incubation were compared in an analysis of variance. The design was a completely randomized factorial; however, due to numerous missing plots, the replications were pooled to form the basic unit of the analysis. Main effects and 2-way interactions were then tested by the 3-way interactions. On the basis of this analysis the length of virus incubation period was significantly increased at lower temperatures, by larger seedlings, and in different flax varieties (P=.99). Varieties CI 1236, 1259 and 1288 were prominent in this respect (Table 17). In addition, there was a trend for a greater range between varieties in length of virus incubation period at 18 and 22.5°C, indicating that if selections were to be made for varieties which have long virus incubation periods, it would be more profitably done at cooler temperatures.

Varieties appear to react similarly whether tested in large greenhouse cages with larger populations of leafhoppers or if inoculated in small numbers under glass chimneys (18). Because of the increased numbers of flax selections derived from infection escapes in previous greenhouse tests and the testing of segregating populations which were derived from crosses between selections of varieties CI 1259 and CI 1236, larger plant numbers were needed than could be used with the chimney method of inoculation. In these experiments 100-125 leafhoppers that were 15-20 per cent AYV viruliferous were fed on 50 flax seedlings per greenhouse insect cage. The percentage of plants with aster yellows from 45 flax lines that have been compared in this manner are presented in Table 18. However, as in the previous technique, only 8 or 9 varieties can be

Table 17. A comparison of the average length of AYV incubation period for 9 flax varieties[a]

Variety CI number	Incubation period in days
1146	14.2
320	15.0
1135 (Marine check)	15.1
1140	16.6
1317	15.6
1164	16.1
1236	16.6
1259	17.0
1288	17.4

[a] Based on an average of approximately 30 plants incubated at 18, 22.5, 24, and 30°C.

[b] Means not enclosed by the same bracket are significantly different (P=.95).

Table 18. Percentage of flax seedlings infected with aster yellows in 1960 greenhouse tests[a]

	Selection from CI number	Percentage of plants infected[b]
1.	1146	82
2.	1146	85
3.	1146	74
4.	1129	92
5.	1129	85
6.	1129	83
7.	1129	76
8.	1129	71
9.	1135 (Marine check[b])	95
10.	1236	63
11.	1236	52
12.	1236	55
13.	1259	59
14.	1259	75
15.	1236	60
16.	1164	67
17.	1140	73
18.	1135	73
(9.)	1135	72
19.	1236	80
20.	1259	71
21.	1236 x 1259 F_2	71
22.	1259 x 1236 F_2	78
(9.)	1135	91
23.	1288	38
24.	1288	53
25.	1288	21
26.	320	26
27.	1146	49
28.	1129	63
29.	1135	52
30.	1236	36
31.	1259	47
(9.)	1135	40
32.	1288	100
33.	1236	79
34.	693	72
35.	1129	79
36.	1164	61

Table 18 (continued)

	Selection from CI number	Percentage of plants infected
37.	320	54
38.	1146	71
39.	1164	60
40.	1164	53
(9.)	1135	88
41.	1336[d]	44
42.	302	52
43.	302	55
44.	1336	50
45.	302	59
(9.)	1135	88

[a] Inoculations were made by feeding 100 to 125 leafhoppers per greenhouse cage, 15-20 per cent AYV viruliferous for 2 days on 50 flax seedlings.

[b] Based on the 60 seedlings, except for selections 19-22 which are based on 40 seedlings.

[c] Usually 8 or 9 flax varieties were compared to Marine as a check at one time.

[d] Flax lines 41-45 were selections from the flax lines of Martin et al. (19).

tested at one time; therefore, variation between tests is sometimes greater than the variation within a given experiment. Nevertheless, in most tests, Marine, the susceptible check variety, had a higher percentage of plants with AYV than the other varieties in the experiment. Some of the most promising lines in these tests appear to be selections from Redwing, CI 302, and CI 1336. At best, however, these lines are not much more resistant to AYV than those which have not been selected.

Observations and studies to account for resistance of flax to AYV.--Observations were made on varietal preference when more than 1 variety was exposed to the insects or on survival of leafhoppers on flax varieties when the varieties were separated by barriers to insect movement. There was never any evidence of varietal preference or any evidence that suggested a rapid mortality of these insects on a particular flax variety with the exception of the insect reaction to selections from the flax variety Redwing (CI 320).

When inoculations were made in the greenhouse cages, the number of insects feeding on a particular variety could easily be counted. Usually there were between 30 and 50 per cent fewer insects feeding on a Redwing selection in a cage than any of the other flax lines. In one comparison between Marine and a selection of Redwing composed of 60 seedlings each, an average of 9.5 insects was observed feeding on Marine whereas 5.2 were feeding at the same time on the Redwing selection. When the virus counts were made, Marine had twice the incidence of aster yellows as compared to the Redwing selection.

Experiments were made also to compare the rate of disease development when insects were fed via celluloic acetate feeding chambers on tillers or main trunks of the flax plants. Several flax selections were compared. In these experiments, almost without exception, symptoms appeared first in the inoculated portion of the plant. Eventually the whole plant became diseased when the main trunks were inoculated. However, when 1 tiller was inoculated, the main trunk was usually healthy, although other tillers might develop symptoms. The line, Iso 419, frequently developed a "shepards crook" in the main trunk and tillers following infection in another tiller (Fig. 9). There were no appreciable varietal differences other than this.

To study the rate at which the virus moves from inoculated leaves into the plant, a leaf feeding technique (Fig. 3) was devised to permit any leaf on the plant to be inoculated with the virus. In this test 5 viruliferous leafhoppers were fed on individual flax leaves for 1 day. Inoculated leaves were then removed immediately following insect feeding, 2 days later, 4 days later, or not at all. From a total of 96 inoculations of 5 different varieties there were 28 successful infections; 11 when the leaves were not removed, 3 when the leaves were removed immediately after inoculation, 6 when the leaves were removed 2 days after inoculation, and 8 when the leaves were removed 4 days after inoculation with AYV. It is possible that varieties might differ in the rate of virus movement into the flax plant; however, with so few successful infections, varietal differences could not be ascertained. Apparently movement of AYV from the leaves into the plant is often

not accomplished immediately following feeding on the insect; the virus may be confined to the leaf for several days following inoculation. Since these experiments were made only with seedlings, no comparisons between age of the leaves were made. It may be that insects feeding on older leaves which no longer contribute plant growth do not serve as suitable vectors.

Evidence for tolerance of flax varieties to AYV

Certain lines of flax selected in greenhouse studies which had longer virus incubation periods and others with shorter virus incubation periods were tested for tolerance to aster yellows at Rosemount in 1959 and 1960. It was believed that the longer virus incubation period in a given variety may indicate depressed virus activity and thereby cause less severe disease. Seed from 10 lines was grown in 8 foot rows in a large screen cage in the field at the Rosemount Experiment Station and inoculated by means of viruliferous insects from the greenhouse. Each flax line was replicated and total yield of seed per row was obtained. Without exception, every flax plant contracted aster yellows. Some lines, however, appeared as though they would yield proportionately better than others because of the difference in severity of the disease (based on the relative number of flowers aborted in the inflorescence). Selections from CI 1259, 1146, and 1236 appeared particularly promising in this test. The lines which yielded the highest were CI 1236, CI 1146, CI 1129, and CI 1259 (Table 19).

Similarly, during the summer of 1960, and selections from CI 693, Marine, 1259, 1236, 1146, and 1129 were compared with and

Table 19. Seed yield in grams from 10 flax varieties grown in screen houses at Rosemount, Minnesota, 1959[a]

Selection from variety, CI number	Yield in grams[b]
1135 (Marine check)	1.0
1140	1.8
1164	3.0
320 (Redwing check)	4.0
1317	4.0
1288	4.5
1259	8.0
1129	8.5
1146	11.0
1236	11.0

[a] Eight foot rows of each of these varieties were grown. Viruliferous insects were introduced after the plants had emerged.

[b] Average of 2 replicates, for all varieties with the exception of Marine and Redwing.

without virus infection. One cage was inoculated with several hundred viruliferous leafhoppers while a similar number of virus-free insects were introduced in the other cage. All of the flax lines were replicated 3 times within each cage, except CI 1146 which was seeded in only 1 row.

When compared to the non-inoculated control, the inoculated plots of selections from CI 1236 and 1146 yielded proportionately higher than those of the other selections (Table 20). These 2 lines were superior also in 1959.

In 1960 a few plants in the inoculated cage were found to be free of virus infection. When progenies from these lines were tested in the greenhouse, they were no more resistant to infection than their predecessors. Apparently these plants escaped infection.

The effect of phosphorus deficiency on the reaction of flax to AYV

During the growing season of 1957, at the Wilderness Farms near Duluth, Minnesota, it was noted by Dr. Wallace Nelson[7] that celery had a low incidence of AYV infection when growing in fertility trial plots low in phosphorus. In view of these results, an experiment was designed to determine if a similar response would occur in flax. Marine flax was seeded (and later thinned to 5 plants) in 4 inch earthen crocks filled with pearlite (a processed volcanic ash) and watered with Hoagland's solution B, supplemented with chelated iron, at the rate of 50 cc per day. This was sufficient to cause runoff. As the plants grew older the amount of solution added at each watering was increased to 100 cc per crock of seedlings.

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[7] Formerly Agronomist, Northeast Experiment Station, University of Minnesota, Duluth; now Superintendent, Southwest Experiment Station, University of Minnesota, Lamberton.

Table 20. Relative performance of 6 flax varieties when inoculated with AYV as compared to non-inoculated[a]

Selection from variety, CI number	Yield in per cent of virus-free control[b]
1135 (Marine check)	8.4
693	9.8
1129	13.2
1259	14.6
1146	24.0
1236	35.2

[a] All flax lines were grown in screened insect houses at Rosemount, Minnesota. Viruliferous insects were allowed to feed on the flax lines in 1 cage and virus-free insects were allowed to feed on the flax plants in the control cage.

[b] Based on the total yield from 3 replicates for all varieties except CI 1146, which was seeded in only 1 long row.

Phosphorus deficiency was created by elimination of the phosphorus from Hoagland's solution. The pH of these 2 solutions was compared before watering and was found to be approximately the same. The crocks were divided into 3 groups. Plants in group 1 were inoculated with AYV by exposure to 100 leafhoppers per 6 crocks for 2 days at the first sign of phosphorus deficiency symptoms (15 days after seeding). The second group was inoculated similarly 4 days later. These plants had pronounced phosphorus deficiency symptoms, i.e., purple discoloration of the leaves and stunting of the plants. The third group was inoculated 3 days later but a complete nutrient solution was added to these plants immediately after insect feeding. Each group or subdivision of the experiments was replicated 3 times. Three weeks after the feeding period, all plants were watered with the complete nutrient solution, since it appeared that the plants with the solution deficient in phosphorus would not survive. Observations were made to determine whether more insects fed on healthy or phosphorus deficient seedlings and on the percentage of seedlings developing AYV.

In none of the 3 experiments was there any indication of preference on the part of the insects for either sufficient phosphorus or phosphorus deficient seedlings.

Phosphorus deficiency greatly reduced plant growth, as would be expected. In addition, there was a reduction in the number of plants infected with AYV in each of the 3 phosphorus deficient treatments (Table 21). When this experiment was repeated, an additional treatment with twice the concentration of potassium was included. Doubling the amount of K in the solution did not

Table 21. Per cent of flax plants with aster yellows when grown in pearlite culture with and without a source of phosphorus[a]

Treatment[b]	Percentage of plants with aster yellows[c]	
	Complete solution	Phosphorus deficient solution
1	13	0
2	47	6
3	53	7

[a] Plants inoculated with 100 insects for 2 days had 25-30 per cent viruliferous insects.

[b] Treatments:
1. Inoculation at the first appearance of deficiency symptoms
2. Inoculation following first deficiency symptoms
3. Inoculation following deficiency symptoms and phosphorus was added immediately after inoculation period to the watering solution

[c] Based on 3 replicates of 5 plants each.

decrease the percentage of plants with aster yellows infection. As previously reported, however, there were considerably fewer virus diseased plants in phosphorus deficient culture solution.

Control of AYV in flax by use of systemic insecticides

Broadcast applications[8].--The effect of 2 systemic insecticides (phorate and Disyston) on the prevalence of M. fascifrons was observed in field plots in North Central Minnesota in 1958 and 1959.

Two farmers' fields of low lime peat soil were selected for these tests at Bagley, Minnesota in 1958. A 10 per cent granular formulation of phorate was applied by broadcast at 6 pounds actual per acre and worked into the soil 1 day before sowing.

In 1959 similar applications of 2 and 4 pounds of phorate and 2 pounds of Disyston were made at Northome, Minnesota, on a sandy loam soil. All treated plots were nearly $\frac{1}{2}$ acre in size and replicated 3 times. The prevalence of plants with aster yellows and the number of leafhoppers in the areas that had been treated with insecticides were compared with the prevalence of aster yellows and number of insects in nontreated areas during the growing season. Yields were taken by harvesting 6 square yards per plot.

There were consistently fewer insects in the phorate treated plots on both farms in 1958 (Table 22). As a result the average incidence of aster yellows in the field was reduced from 7.5 to 1.5 per cent.

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[8] Work done on these experiments was in cooperation with Dr. Allen Peterson, Department of Entomology and Economic Zoology, University of Minnesota.

Table 22. Periodic counts of leafhoppers (M. fascifrons) present in ½-acre plots from 2 farmers' fields treated with 6 pounds of phorate for insect control at Bagley, Minnesota, 1958.

Date	Average number of leafhoppers per 50 sweeps[a]			
	Swenson Farm		Darst Farm	
	Phorate	Control	Phorate	Control
June 15				
Adults	0.3	1.7	0.0	0.0
Nymphs	0.0	0.0	0.0	0.0
July 12				
Adults	7.1	51.3	8.7	22.7
Nymphs	0.0	0.7	0.0	0.0
August 5				
Adults	22.0	57.0	13.0	38.3
Nymphs	0.0	12.0	0.0	26.7

[a] Average of the 3 replications.

Similarly, in 1959 both rates of phorate markedly reduced the number of leafhoppers and percentage of plants with aster yellows (Tables 23 and 24). Disyston was less effective than phorate in controlling the insect vector and the incidence of aster yellows was greater in these plots.

No differences in yield were detected in 1958; however, in 1959 all treated plots yielded higher than the control (Table 24). Although Disyston was less effective against the leafhopper, soil treated with Disyston produced the higher yield indicating that high rates of phorate may be phytotoxic.

Seed treatment and placement of systemic insecticides.-- Control of both insects and virus has been achieved by broadcast application of systemic insecticides. However, from a cost standpoint, this method is not acceptable because of quantity of insecticide needed.

The question was asked whether smaller quantities of insecticides applied as a seed coating (seed treatment method) or as a band application (a strip of insecticide placed in the soil below the flax seed) could be used as a control of aster yellows in the field.

During the summer of 1960 an experiment was made in the field at St. Paul to test these methods. A granular formation of phorate, 10 per cent actual, was used in the band treatment and a formulation in a carbon base, 44 per cent actual, was applied to seed by means of a methylcellulose slurry. The band applications were placed $\frac{1}{2}$ inch below the flax seed in the field using 0.5, 1.0,

Table 23. Periodic counts of leafhoppers (M. fascifrons) present in ½-acre flax plots treated with 2 systemic insecticides at Northome, Minnesota, 1959

Date	Average number of leafhoppers per 50 sweeps[a]			
	Phorate		Disyston	Control
	4 lb/A	2 lb/A		
June 12				
Adults	28.5	43.5	104.0	61.5
Nymphs	0.0	0.0	0.0	0.0
June 26				
Adults	24.3*	33.3*	123.3	98.3
Nymphs	0.0	0.0	1.8	5.0
July 24				
Adults	71.8*	78.5*	176.0*	348.3
Nymphs	9.3*	27.5*	115.0*	685.8

[a] Average of the 3 replications.

* Significantly lower than the control (P=.95). On July 24 the 2 phorate treatments were also significantly lower than the Disyston treatment.

Table 24. Percentage of aster yellows and yield of flax in $\frac{1}{2}$-acre plots treated with systemic insecticides for the control of the six-spotted leafhopper

Treatment	Percentage of plants infected with aster yellows[a]		Yield in bushels per acre
Phorate			
4 lb/A	4.8[b]	a	14.5[c]
2 lb/A	9.0	a b	16.9
Disyston			
2 lb/A	11.9	a b	17.0
Control	18.8	b	12.6

[a] Average infection from 300 plants selected in each of 3 replications.

[b] Means not followed by the same letter are significantly different (P=.95).

[c] No significant differences.

and 1.5 pounds of active phorate per acre, whereas the seed was treated with 0.5, 1.0 and 1.5 pounds of phorate per 100 pounds of seed. The experiment was a split-plot design with treatments randomly allotted to whole plots except that the 3 treatments involving band applications and 1 control were grouped together in 1 randomly selected half of each replicate, and the 3 seed treatments and another control were allotted to the other half. In the analysis of variance the whole plots were treated as completely randomized. Sub-plots were 3 varieties, Arny, Marine, and Redwing, each seeded at the rate of 100 seeds per 6-foot row. Varieties were seeded 1 foot apart whereas whole plots were separated by 4 feet. The experiment was replicated 4 times. Approximately half of each plot was caged with insect cages 3 weeks after seeding. One hundred AYV viruliferous leafhoppers were introduced into the cages in 2 replicates 3 weeks after seeding. The other 2 replicates were inoculated similarly 2 weeks later. Cages were removed 3 weeks after the insects were introduced. Since there were no statistically significant differences between the 2 dates of inoculation, all data presented are the mean of 4 replicates.

Notes were taken on stand, insect kill, phytotoxicity of the insecticide, and per cent of flax plants with aster yellows infection.

There was a reduction in stand of nearly 15 per cent in the 1.5 pounds per acre of phorate treatment, but no appreciable reduction in stand due to any of the other treatments. In the high

phorate treatment, a slight firing of lower leaves appeared 2 to 4 weeks following seeding, but subsequent growth did not appear to be damaged.

Insects in cages over plots treated with the band application of phorate were all killed within 2 days following their introduction, whereas there appeared to be little, if any, insect mortality in plots with seed treatment of phorate or the control plots.

Significant reductions in the percentage of aster yellows infection were obtained in all 3 of the band applications of phorate. There was some control of AYV by treating the seed with 1.5 pounds of phorate per 100 pounds of seed (Table 25). At the plant populations used, rate for rate, the dosage used in the band application was about 10 times greater than the seed treatment on an individual plant basis. Therefore, the 1.5 pounds of phorate per 100 pounds of seed equals, on an average, 1/3 of the dosage of the 0.5 pound per acre band treatment. It appears on the basis of this test that the most efficient level of insecticide may lie somewhere between 1/4 to 1/2 pound per acre or perhaps 2 to 4 pounds of insecticide per 100 pounds of seed. It should be noted that this insecticide was still available in the plant as long as 5 weeks following seeding.

Surprisingly, there was a definite difference in the incidence of virus in the flax varieties compared. Redwing had significantly fewer virus diseased plants than did either Arny or Marine.

Table 25. Percentage of flax plants in field plots with aster yellows after treatment with phorate for vector controls[a]

Rate of phorate application	Percentage of plants with virus[b]
A. Band application in pounds per acre[c]	
1.5	15 a[d]
1.0	12 a
0.5	16 a
None	63 bc
B. Seed treatment in pounds per 100 pounds of flax seed[e]	
1.5	44 ab
1.0	53 ab
0.5	89 c
None	86 c

[a] One hundred viruliferous insects introduced in cages placed in each plot.

[b] Average of 4 replicates.

[c] Phorate applied to the soil in a strip directly below the flax.

[d] Treatments not followed by the same letter were significantly different at the 1 per cent level.

[e] Phorate applied to the seed in a slurry of methylcellulose.

DISCUSSION AND CONCLUSIONS

In these investigations the effects of 2 virus diseases of flax, aster yellows and crinkle, were studied. The symptoms and transmission of each virus as well as the symptoms caused by double infections of BDV and AYV and the dual transmission of the viruses by M. fascifrons are described. Observations on yield losses of flax caused by these virus diseases, the effect of temperature on disease development, and the influence of plant stand on the incidence of virus infection in the field are presented. The control of aster yellows through development of resistant varieties and by vector control with systemic insecticides were studied also.

The symptoms caused by these viruses are distinctly different from each other. Aster yellows is conspicuous in a commercial flax field due to the yellow-green inflorescence in a principally blue background when flax is in bloom. Crinkle, on the other hand, is difficult to detect unless a careful examination for small enations on the plant leaves or sepals is made. Crinkle is most easily observed when the diseased flax plants are in a pre-bloom stage of development. Leaves in the apical whorl are commonly twisted and curled in a crinkled appearance, hence the name. Double infections of AYV and BDV commonly cause symptoms different from either virus alone, particularly the hypertrophy of diseased plant apices.

Both viruses were transmitted to flax by dodder (Cuscuta sp.) and the six-spotted or aster leafhopper (M. fascifrons). Not

only did few insects recover AYV from diseased flax but those insects which did recover AYV had long virus incubation periods, indicating that the amount of virus acquired was low (17). The six-spotted leafhopper proved to be an efficient vector for inoculating flax with AYV but not efficient in recovering AYV from diseased flax. The six-spotted leafhopper was likewise an efficient transmitter of BDV, the virus causing crinkle. However, it appeared that BDV was recovered from flax more readily than AYV by the insect vector.

Crinkle symptoms develop sooner than aster yellows in the field, probably due to a shorter virus incubation period at cooler temperatures. Also there is a possibility of easier acquisition of BDV by the leafhopper, and a shorter incubation period of the virus in the insect. In addition, oats and barley which are excellent hosts for both BDV and the vector (1, 2) are widely grown throughout the commercial flax areas. These factors, when taken into consideration, strongly indicated that crinkle should be more prevalent in a given year than aster yellows. In 1957, at St. Paul, Minnesota, in an experiment with 2 seeding dates, the incidence of crinkle increased from 9 per cent in the first seeding to 39 per cent in a later seeding of flax, while the incidence of aster yellows increased from 22 to only 26 per cent. Although repeated efforts in this experiment were made to detect all crinkle plants, it is possible, due to the inconspicuous nature of crinkle in older plants, that a few diseased plants were not detected; however, this would have been true also for the second seeding and the relative range in incidence of virus infection between the 2 seedings was probably not influenced, although the total number of diseased plants may have been greater than presented.

Both viruses can simultaneously infect flax; however few plants infected with both AYV and BDV were found in the field. Again it is interesting, but probably not economically significant, that both viruses can be transmitted by a single insect. Even when the six-spotted leafhoppers were exposed to flax infected with both viruses, an individual insect, in greenhouse trials, recovered only 1 of the 2 viruses. Insects transmitting both viruses were obtained when insects were fed on separate plants, each infected with 1 of the 2 viruses. This further suggests that dual virus transmitting insects in the field will be rare.

Both virus diseases were found to significantly reduce flax yield. There was a reduction in number of seeds per capsule and the number of seeds per boll as a result of infection by either virus. Earlier infections of AYV caused greater yield losses in the field than later infections. The data obtained for yield loss due to crinkle were determined from plants diseased in the pre-bloom state, and no data comparing time of BDV infection to yield loss were made.

Underlying these prospective disease controls, particularly the use of systemic insecticides, is the question of disease forecasting. Extensive studies by Chiykowski (5) and others (16, 26) have pinpointed spring migration habits of the six-spotted leafhopper. Their studies demonstrated that the number and direction of the migrating insects varies from season to season, but in general insects are carried northward from the winter grain fields of Missouri and Kansas by the warm southern winds each spring. The number of

migrating leafhoppers and local overwintering insects present in a given area and the time at which they appear are not the only important considerations in aster yellows or crinkle of flax disease forecasting. One other factor, the number of insects within the population which are carrying the virus, must be determined. For each year since 1957 the early arriving insects have been indexed individually for the percentage of these insects transmitting AYV and for both AYV and BDV from 1959 to 1961. In 1957, over 10 per cent of the insects transmitted AYV, the year of the aster yellows epidemic. Since then a much lower percentage of leafhoppers transmitted AYV; usually less than 2 per cent and less than 1 per cent in 1960. Observations similar to these have been made recently by Westal et al. (26). In none of the years since 1957 has there been an aster yellows epidemic, although a fairly large number of insects was present early in each season, particularly in the spring of 1960 and 1961.

A number of different control measures for aster yellows were tried. At the present time, control of aster yellows by means of introducing resistant or tolerant varieties could not be recommended for 2 reasons. First, the level of resistance to disease is not high, and second, those varieties most resistant or tolerant are not acceptable from an agronomic standpoint. For example, Redwing and CI 1336, both well adapted to Minnesota growing conditions, are susceptible to rust and wilt and to wilt, respectively. The variety CI 302 is susceptible to flax wilt, pasmo and lodging and in nearly all other respects it is poorly adapted to cultivation in Minnesota.

Flax varieties were found which had a significantly longer virus incubation period than others. Whether or not this criterion is suitable as a basis for selection of tolerant varieties is questionable. Varieties CI 1236 and 1259, 2 flax lines which had long virus incubation periods, were found to yield proportionally better than several other varieties when grown under severe disease conditions; however CI 1146, a variety which had a short virus incubation period, yielded proportionally better also than most of the other varieties in these tests.

Obviously, insecticides, if sufficiently effective in eliminating or depleting a leafhopper population, will reduce the incidence of virus. Unfortunately the cost of broadcast application, together with the fact that high rates of phorate may be phytotoxic, is too expensive as a means of controlling outbreaks of aster yellows in flax even if the applications were made in a year in which the disease was accurately forcasted. Band or strip applications which have been effective in reducing aster yellows of potatoes commercially by vector control (27) may also provide adequate control of aster yellows of flax in commercial fields.

SUMMARY

1. A strain of aster yellows virus (AYV) was found to cause aster yellows disease of flax.

2. A new virus disease of flax, crinkle, caused by the oat blue dwarf virus (BDV) was reported.

3. The symptoms of aster yellows in flax, although varying with stage of growth, generally are phyllody and yellowing of the flowers. Most commonly in the field, aster yellows infection is partial; i.e., there are both normal and diseased flowers within the inflorescence.

4. BDV infection of flax is difficult to detect because the only symptoms are small enations on the lateral veins of the leaves and sepals and slight dwarfing.

5. Infection by both AYV and BDV in flax will generally result in a greatly enlarged or hypertrophoid apex of the plant, a symptom not typical of either virus alone.

6. Both AYV and BDV were easily transmitted to flax by Macrosteles fascifrons (the six-spotted leafhopper) although recovery of either virus from diseased flax by the leafhopper was inefficient when compared to virus recovery from other diseased hosts.

7. Individual insects were obtained which transmitted simultaneously both AYV and BDV to flax.

8. Early field infection of both AYV and BDV in flax significantly reduced the yield.

9. Early maturing flax varieties were less severely affected by AYV than those varieties which matured later.

10. Higher planting rates and earlier seeding both reduced the incidence of aster yellows and crinkle in the field.

11. Incubation period of AYV in flax is about half as long at 30°C as at 18°C, however the incubation period of BDV is the same length at both 18° and 30°C.

12. Flax lines less susceptible to aster yellows than others have been found. Selections from varieties CI 302, CI 1236, CI 1259, CI 1336, and Redwing are the most promising.

13. Phosphorus deficient flax plants have a lower incidence of aster yellows than normal plants.

14. Systemic insecticides, phorate and Disyston, when applied at 4 pounds per acre by broadcasting can reduce leafhopper populations and thereby control aster yellows. Soil band applications of phorate, $\frac{1}{2}$ to $1\frac{1}{2}$ pounds per acre, have effectively controlled the vector and reduced the incidence of aster yellows virus from nearly 80 per cent in the control plots to less than 10 per cent.

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